Investigations of HRC®-Stimulated Bioreduction of Cr(VI) at Hanford 100H

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REGENESIS

http://www-esd.lbl.gov/ERT/hanford100h/

NABIR

Abstract

Hypothesis: Lactate (Hydrogen Release Compound—HRC™) injection into chromium contaminated groundwater through an injection well will cause indirect or direct bioreduction of chromate [Cr(VI)] and precipitation of insoluble species of [Cr(III)] on soil particles, probably catalyzed at oxide surfaces, at the field scale.

Objective: Assess the potential for immobilizing and detoxifying chromium-contaminated groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford Site's 100-H Area field site.

Types of Research: A three-well system (injection well and upgradient and downgradient monitoring wells) was used for conducting the in situ biostimulation and monitoring. To assess the pre- and post-injection test groundwater conditions, we used an integrated monitoring approach, involving hydraulic, geochemical, microbial, and geophysical techniques and analytical methods, as well as conducted five Br-tracer injection tests and four pumping tests (concurrently with the Br-tracer tests).

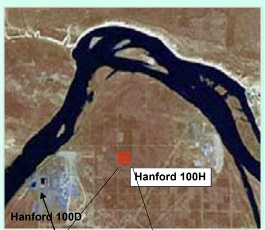
Groundwater biostimulation was conducted by injection of 40 lbs of ^{13}C-labeled HRC into the injection well (over the depth interval from 44-50 ft) on 8/3/2004, followed by low-flow pumping (1.2 to 2.5 l/min) through the downgradient well (to ensure capture of groundwater flow lines passing through the injection well) for 27 days.

Main Results: Although the total microbial population in sediments is relatively low (<10^5 cells g-1) under background conditions, which is likely insufficient for direct enzymatic Cr(VI) reduction, several types of bacteria, e.g., Bacillus/Arthrobacter and Geobacter, are present in the Hanford sediments, which are known to reduce or sorb hexavalent chromium.

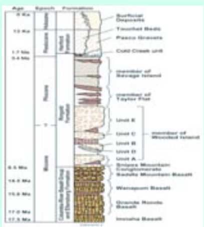
The HRC injection stimulated microbial cell counts to reach the maximum of 2×10^7 cells g^{-1} 13-17 days after the injection, and generated highly reducing conditions. Geochemical and isotopic observations confirmed microbial metabolism of HRC.

The Cr(VI) concentration in the monitoring and pumping wells decreased below drinking water minimum contaminant limits and remained below background concentrations even after 1.5 years, when redox conditions and microbial densities had returned to background levels. Fe(II) levels have remained high and may account for the continued reduction of Cr(VI).

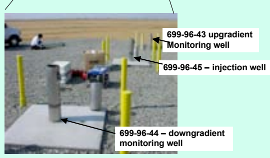
I. Hanford 100H Site Characterization



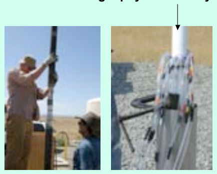
The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7.2H_2O$)



Lithological Column

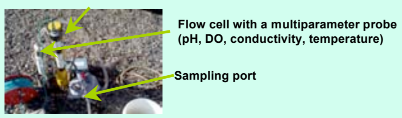


Access tube for geophysical survey

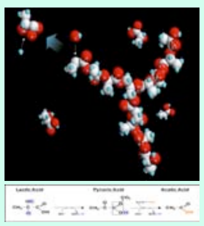


Groundwater sampling and packer inflation using argon gas

Flow cell with Br sensor

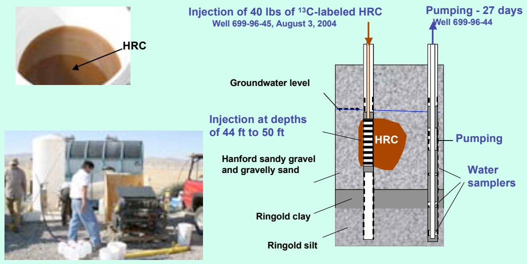


II. Lactate/Polylactate (HRC™) Properties



- Polymer of lactic acid (glycerol polylactate)
- Fermented by indigenous microorganisms to produce H_2, an electron donor
- Slowly releases lactic acid to stimulate anaerobic bacteria and ferment the lactic acid to gain carbon and energy

III. Pilot Field Experiment of Groundwater Biostimulation Using HRC Injection



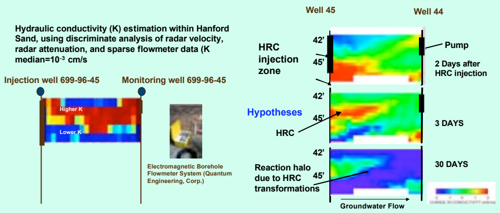
- All in all 11 gal of water were used as a primer to fill the injection hose before the injection, dilute HRC, and as a chaser after the HRC injection.
- Br breakthrough occurred 7 days after the injection, and the maximum was reached 11 days after the injection.
- Microbial cell counts reached the maximum 13-17 days after the injection.

IV. Post-HRC Injection Monitoring

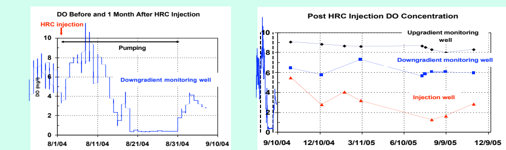
IV.1. Geophysical Observations of HRC Distribution and System Transformations

• Tomographic radar and seismic datasets were collected prior, during, and after HRC injection and then interpreted in conjunction with laboratory results.

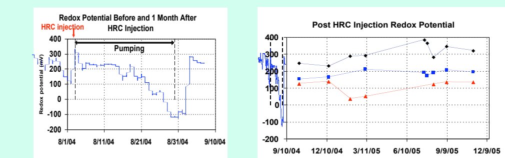
• The electrical response could be associated with the disappearance of nitrates and sulfates, the increase in dissolved gas (H_2, CO_2, H_2S), or the increase in amorphous precipitates (sulfides).



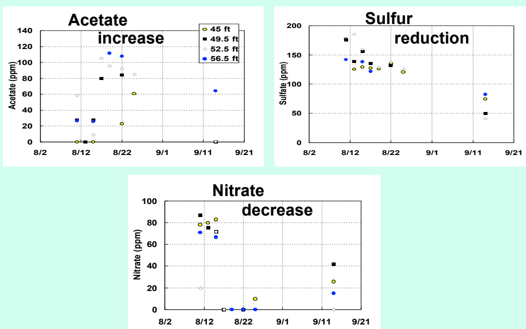
IV.2. Geochemical Evidence of Biostimulation

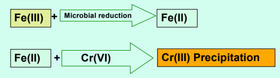


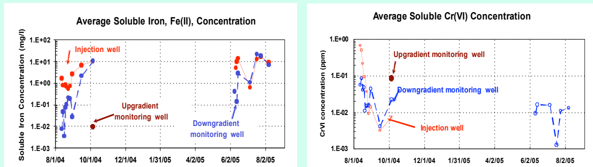
After the HRC injection, dissolved oxygen (DO) dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)



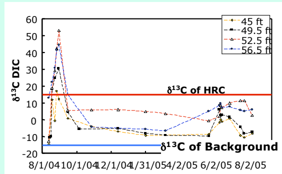
After HRC injection, redox potential dropped from 240 to -130 mV



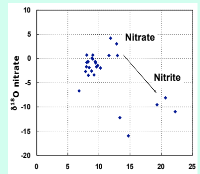




IV.3. Isotopic Evidence of Biostimulation



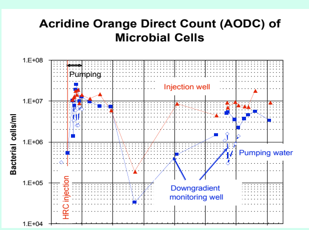
δ^{13}C of dissolved inorganic carbon (DIC) is byproduct of HRC metabolism



Decrease in δ^{18}O and increase in δ^{15}N is evidence of denitrification

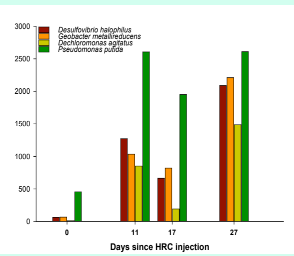
IV.4. Microbial Analyses

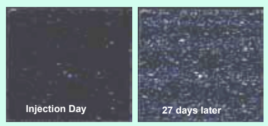
- Phospholipid fatty acid analyses (PLFA)
- Terminal restriction fragment length polymorphism (T-RFLP) with primers for Fe and sulfate reducers, and nitrate dissimilatory reactions;
- Live/dead direct counts;
- TEA, ED, DOC, DIC, CO2, O2 Limiting nutrients, e.g., N, P, S, Fe;
- Nitrogen and oxygen isotope ratio;
- ^{53}Cr/^{52}Cr ratios
- Clone libraries
- 16S rDNA GeneChip
- Direct rRNA analysis by microarray
- Novel PCR independent analysis of microbial communities (Bacteria and Archaea)



- Archaea
  - Only Crenarchaeotes (non-thermophilic) detected
    - Dominated by one type with no cultured relative.
- Bacteria initial enrichment of denitrifiers
  - *Fulvimonas, Pseudomonas, Hyphomicrobium, Acidovorax, Aquaspirillum, Thauera, Azoarcus, Comamonas, Dechloromonas, Clostridium.*

Major increase in microbial organisms after the HRC injection



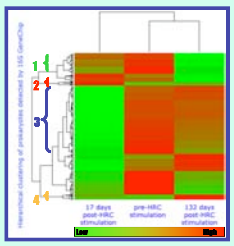


16S rDNA Clone library & qPCR

Sequencing at JGI
⇩
1000+ clones
⬇
Phylogenetics
⬇
qPCR
⬇
Metagenomics?

D. Vulgaris (direct fluorescent antibody)

Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation

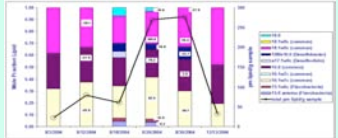


Group1 organisms decline:
Pseudomonas, Burkholderia (Denitrifiers)
Acidithiobacillus, Thiothrix (Sulfur oxidizers)
Leptothrix (Iron oxidizer)

Group2 organisms increase then decline:
Acidovorax, Thauera (denitrifiers)
Flavobacteria (aerobes, use glycerol)

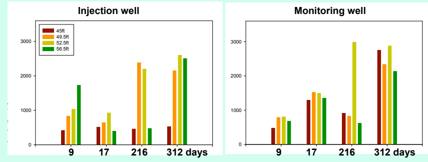
Group3 organisms decline then return:
Mainly oligotrophic bacteria

Group 4 organisms increase in late stages:
Legionella, Chlamydophila, Flectobacillus.

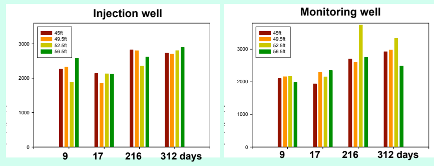


Results of ^{13}C Phospholipid Analysis:
- General bacterial biomarkers indicate rapid enrichment in ^{13}C
- ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)
- ^{13}C polylactate used as spike it is not esterified to glycerol backbone
  - it is released and consumed more rapidly
- Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment with ^{13}C.
- *Flavobacteria* do NOT typically utilize lactate, but may use glycerol (backbone, unlabeled)

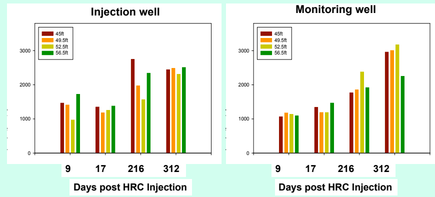
Presence of Euryarchaeota (Methanogens) indicate methanogenic conditions after HRC injection



Presence of Desulfovibrio may help maintain reducing conditions by producing H_2S



Presence of Geobactermay help maintain reducing conditions by producing Fe(II)



IV. Key Findings

• Several types of bacteria are present in the Hanford sediments, including *Arthrobacter, Oxalobacter, Sporomusa* and *Pseudomonas* species. Under background conditions, the total microbial population is <10^5 cells g^{-1}.

• Pilot field-scale HRC biostimulation of the groundwater shows that microbial cell counts reached the maximum of 2×10^7 cells g^{-1} 13 to 17 days after the injection and continued to increase for the first 6 weeks, followed by the decrease in the microbial diversity. The HRC injection caused highly reducing conditions: DO dropped from 8.2 to 0 mg/l and redox potential from 240 to -130 mV. Despite the fact that DO and nitrate began to return to background concentrations two months after HRC injection, bacterial densities remaining high (>10^7 cells/ml).

• Geophysical investigations show that HRC products (such as lactic acids) injected into groundwater can be detected using radar and seismic survey, and that even small variations in hydrogeological heterogeneity may influence the distribution of the amendment and its products.

• δ^{13}C ratios in dissolved inorganic carbon confirmed microbial metabolism of HRC. Increases in carbon isotope ratios of DIC in Well 44 coincide with increases in bromide, chloride and acetate and decreases in nitrate.

• Hydrogen sulfide production was first observed after about 20 days post-injection, which corresponds with the enrichment of a *Desulfovibrio* species (sulfate reducer).

• Cr(VI) concentrations in the monitoring and pumping wells decreased significantly and remained below up-gradient concentrations even after 6 months, when redox conditions and microbial densities had returned to background levels. High levels of Fe(II) may cause the continued reduction of Cr(VI).

• Ongoing research are aimed at the evaluation of sustainability of chromium biostimulation, including the field observations and modeling of Cr(III) reoxidation, the evaluation of the HRC amount and distribution in the subsurface, and the need for pumping.

Selected Publications:

Hazen, T.C. and H. H. Tabak, Developments in bioremediation of soils and sediments polluted with metals and radionuclides: 2. Field research on bioremediation of metals and radionuclides, *Reviews in Environmental Science and Bio/Technology*, 4:157–183, 2005.

Hazen, T.C.. D. Joyner, S.Borglin, B.Faybishenko, J.Wan, T.Tokunaga, M.Conrad, C.Rios-Velazquez, J.Malave-Orengo, R.Martinez-Santiago, M.Firestone, E.Brodie, PE. Long, A.Willet, and S.Koenigsberg, Functional Microbial Changes During Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) in Hanford 100H Sediments, Abstract, Fourth International Conference "*Remediation of Chlorinated and Recalcitrant Compounds*", May 24-27, 2004.

Linde, N., S. Finsterle, and S. Hubbard, Inversion of hydrological tracer test data using tomographic constraints, *EOS Spring Supplement*, Montreal, Canada, May 2004.

Tokunaga TK., J.M Wan, T.C. Hazen, E. Schwartz, M.K. Firestone, S.R.Sutton. M. Newville, K.R. Olson, A. Lanzirotti, and W. Rao, Distribution of chromium contamination and microbial activity in soil aggregates, *Journal of Environmental Quality*, 32(2):541-549, 2003.

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